APPLICATION FOR UNITED STATES PATENT

in the name of

Graham Williams, Joss Delves-Broughton, John Fragher and Diane Salemla

for

Baked product with increased shelf life and process for increasing the shelf life of baked products

Fish & Richardson P.C.
1425 K Street, N.W.
11th Floor
Washington, DC 20005-3500
Tel.: (202) 783-5070
Fax: (202) 783-2331

ATTORNEY DOCKET:

17086-002001 / A3771PUS

Baked product with increased shelf life and process for increasing the shelf life of baked products

TECHNICAL FIELD

The present invention relates to a non yeast-leavened fine bakery product with increased shelf life and to a process for increasing the shelf life of intermediate and high moisture fine bakery products.

BACKGROUND

Many industrially produced baked goods emerge from the baking process with a surface that is essentially sterile but post bake handling can quickly lead to fungal surface contamination as a result of exposure to air borne contaminants as well as equipment contact. Following surface contamination, many baked goods are then very vulnerable to surface mould spoilage, the severity of which is linked to factors such as the degree of contamination, the moisture content of the product and the storage conditions. Baked products with a relatively neutral pH, high moisture content and high water activity such as cakes, muffins, waffles, and tortillas are particularly prone to rapid spoilage from a variety of moulds, principally *Penicillium* and *Aspergillus* species. Manufacturing good tasting, high moisture products with a long mould free shelf life presents a constant and ongoing technical challenge to the baking industry.

Various methods have been adopted in an attempt to achieve the required shelf life. These include addition of humectants to reduce the water activity, addition of chemical mould inhibiting preservatives such as propionates or sorbates limiting the availability of oxygen via modified atmosphere packaging and active packaging containing oxygen scavengers or providing a saturated ethanol headspace in the pack using sachet or strip inserts containing ethanol. The chemical preservatives such as sorbate and propionate are most effective at low pH so acids are often added in combination with these preservatives to reduce the pH of the baked product and hence improve the effectiveness of the added preservative.

Addition of acids, chemical preservatives and humectants can affect the taste and quality of the product and their use is often a compromise between achieving the best tasting product and the longest possible shelf life. Preservation based on packaging systems rely very much on pack integrity and even the best systems can suffer shelf life failures due to pack damage or seal failures and hence loss of pack integrity. Thus, there remains the technical problem of providing an efficient preservation system, which will not adversely affect the taste of baked goods.

Natamycin is a polyene macrolide natural anti-fungal agent produced by fermentation of the bacterium *Streptomyces natalensis*. Natamycin (previously known as pimaricin) has an extremely effective and selective mode of action against a very broad spectrum of common food spoilage yeasts and moulds with most strains being inhibited by concentrations of 1-15 ppm of natamycin.

Natamycin has been used for many years in a large number of countries throughout the world as an authorized preservation treatment for cheeses and certain meat products such as dried sausages. Despite this long-term use, the development of resistant strains has not been reported to date unlike the chemical organic acid sorbate and propionate preservatives for which a number of resistant yeasts and moulds have been detected and reported. Some species of *Penicillium* mould are even able to degrade and metabolise sorbate.

Natamycin is much less soluble in water than the chemical organic acid preservatives with its maximum solubility being around 40 ppm. In practice this means that when applied to the surface of the cheese or sausage, natamycin shows very limited diffusion and tends to stay on the surface of the food. Natamycin is active over a wide pH range and unlike the organic acid preservatives it is not dependant on a low pH acidic environment to show good anti-fungal activity. The effectiveness of natamycin at very low application levels on cheese and sausage has not been reported to have any adverse quality or flavour impact on the products.

Although natamycin has been used for a long time on cheese and on sausages, there is very little reported on the use of natamycin for other types of food. According to a review article in the 1974 edition of the Encyclopaedia of Food Technology by A.H. Johnson and M.S. Peters, natamycin (pimaricin) is permitted in several countries as a food additive and it is either added into the food (e.g. orange juice, wine) or the food is dipped, soaked or sprayed with aqueous natamycin (e.g. cheese, sausage, fruit).

In the U.S. the direct addition of natamycin into tortilla dough before baking is allowed. Tortilla dough is leavened without yeast and therefore adding natamycin into the tortilla dough is possible. In yeast leavened doughs, natamycin cannot be used since the natamycin would kill the yeast. Because of this limitation of using natamycin in yeast leavened bakery, natamycin seems to have been tested on the surface of yeast leavened bread. Thus, the review in the above mentioned 1974 Encyclopaedia also mentions that "rye and white bread were well protected when their surfaces were sprayed with a solution of 100 –500 ppm pimaricin". No specific results are quoted to support the "well protected" comment and no reference is quoted for this work. No target levels for natamycin / pimaricin on the surface of the bread are given, no method of spraying and no shelf life targets are mentioned. In a later update of this review article for the same Encyclopaedia there was no mention of this bakery work. The review also makes similar vague references to treating the surface of uncooked doughs and to direct addition at 25 – 50 ppm in fillings for cakes and pies.

The direct addition of natamycin into icings and fillings of cakes is described also by J. Tichá in Mlynsko-pekarensky promysl, 7/1975, pp 225-228, as being effective in preventing the growth of moulds and yeasts for about 14 days. The article concludes that natamycin in admixture with lactose is useful to preserve curd fillings, icings and butter creams.

The addition of natamycin to protect the fillings of Cantonese mooncakes and pastry is allowed according to Chinese legislation. However, the mooncakes themselves often have a fairly low

water activity and are thus not as prone to spoil as the fillings, which are often made of easily perishable foodstuffs.

Many fine bakery products are required to have a very long shelf life. Intermediate and high moisture fine bakery products such as muffins, tortillas, waffles, sponge cakes and the like are usually packaged and kept at ambient temperatures on the shelf for 2 to 10 weeks and sometimes longer. In contrast to this, yeast-leavened baked goods, such as bread, tend to go stale in a much shorter period and the shelf life of most bread is normally not above two weeks.

The high water content of many fine bakery products makes them very sensitive to spoilage due to mould and yeast growth. This is especially true when the water activity of the product a_w is 0.8 or more, especially 0.85 or more. In an attempt to protect soft intermediate moisture and high moisture baked goods from mould growth, the present inventors added natamycin to tortilla dough, as allowed by the U.S. regulations, and to the pastry dough and egg glaze of Chinese mooncakes pre-baking. However, surprisingly, the attempts failed and did not result in any significant improvement of the shelf life of the baked goods. It was found that incorporating natamycin within the baked good was unsuccessful despite the fact that natamycin levels, which would normally be considered effective against yeasts and moulds, could still be detected in the goods after the baking process.

In two separate trials where natamycin (as Natamax) was incorporated into tortillas, the control of surface mould growth during shelf life was not achieved. Analysis of the mouldy tortillas from both trials showed that natamycin was still present within the tortillas at levels between 14.0 and 28.0 ppm. These relatively high levels of residual natamycin would normally be expected to show good control of mould growth indicating that the natamycin present within these tortillas was not biologically available at the surface of the product where it is required to be effective.

Consequently, natamycin could not be used in the non yeast-leavened baked products in the way the person skilled would normally and preferably have applied it, i.e. by direct addition of natamycin to the ingredients prior to baking. There thus remained the problem of how to increase the shelf life of fine bakery products having a high water activity and tendency to mould growth whilst optimising on desirable product characteristics such as pH and taste.

Documents cited in this text ("herein cited documents"), as well as each document or reference cited in each of the herein-cited documents, and all regulations, manufacturer's literature, specifications, instructions, product data sheets, material data sheet, and the like, as to each product mentioned in this text, are hereby expressly incorporated herein by reference.

SUMMARY

The present invention is based on the realization that natamycin can still help to overcome preservation problems of intermediate and high moisture fine bakery goods provided that it is applied to the surface of the baked products after baking. The relatively high moisture content in the baked products ascertains that an effective amount of dissolved natamycin will continuously be present to combat yeast and mould growth on the surface of the baked products.

Thus, the present invention provides an intermediate or high moisture non yeast-leavened baked product having a water activity a_w >0.8, the surface of said bakery product having deposited thereon an effective amount of natamycin which is sufficient to keep said product mould free when packaged for a storage time of 2 weeks or more at ambient temperature.

The present invention also provides a process for increasing the shelf life of fine bakery products, comprising providing a baked fine bakery product having a water activity a_w >0.8; spraying the outer surface of said baked product with natamycin to deposit an effective amount of natamycin thereon; packaging said sprayed baked product into a protective envelope; and storing said packaged product at ambient temperature. The natamycin deposited on the surface of said bakery product is provided in an amount which is effective in keeping said product mould free even when stored for 2 weeks or more.

The fine bakery product of the invention is preferably a non yeast-leavened fine bakery product having a water activity a_w between 0.8 and 0.95, preferably between 0.80 and 0.90, most preferably between 0.85 and 0.90. The preferred long shelf life fine bakery products protected by the invention are selected from muffins, waffles, tortillas, sponge cakes and the like baked fine bakery products.

The increase of mould-free shelf life achieved by the present invention is of immense significance to the baking industry and will allow the replacement of chemical preservatives such as propionate, benzoate and sorbate with natamycin, a less toxic, tasteless and naturally occurring alternative. The belief that natamycin is not effective in baked goods because it is not effective when incorporated into the food prior to baking, has been shown to be false and the problem has been overcome by the present invention by spraying the natamycin onto products post baking. Even though this requires prospective users to invest in suitable spraying equipment that can deliver an even natamycin application to all surfaces of bakery products, the advantages of natamycin are seen to outweigh the disadvantages of its use.

Without wishing to be bound by any theory, it is believed that the lack of natamycin efficacy noted when the natamycin was included directly into the ingredients of the fine bakery products was caused by a combination of 1) heat degradation of surface natamycin and 2) formation of a surface crust during baking that prevented the natamycin within the baked good from reaching the surface.

DETAILED DESCRIPTION

The lack of effectiveness that was seen when natamycin was incorporated into non yeast-leavened high water activity bakery products prior to baking led to the idea for the novel alternative solution of applying natamycin as a water based suspension precisely sprayed direct onto the exposed surfaces of the high water activity fine bakery product after the baking process. This novel approach targets a sufficient concentration of available natamycin directly at the surface of the baked

product where the fungal contamination occurs. Any problem of losses during the baking process and availability on the surface has been overcome.

Consequently, the invention provides a fine bakery product the shelf life of which has been increased by the use of natamycin on the surface thereof. The preferred fine bakery products are non yeast-leavened products intended for a long shelf life and having a moisture content which makes them susceptible to surface spoilage by moulds and yeasts. Such fine bakery products are especially intermediate or high moisture baked products having a water activity a_w >0.8, preferably 0.85 or more. A moisture content of 0.8 to 0.85 is regarded herein as an intermediate moisture content, while a moisture content above 0.85 is regarded as a high moisture content. Typical examples of such fine bakery products are cakes, muffins, waffles, tortillas and similar high water activity baked products.

The surface of the fine bakery product of the invention has deposited thereon an effective amount of natamycin which is sufficient to keep the product free of mould and yeast growth even though the product is packaged and stored for a time of 2 weeks or more. The effective amount of natamycin on the surface of the finished product is between 1 – 10 µg per cm^2 and it is sufficient for keeping the baked product mould free for 3 to 10 weeks, preferably for 6 to 10 weeks, or even longer, when the product is stored at ambient temperature, which usually ranges between 15 and 30°C and which more often is from 18 to 25°C.

When the process of the present invention is operated, the outer surface of a baked fine bakery product having a water activity a_w >0.8 is sprayed with natamycin. The spraying should be performed so that a preservatively effective amount of natamycin is deposited on the exposed surface of the product. Typically, the natamycin is sprayed onto the exposed surface in the form of an aqueous suspension containing natamycin as dissolved natamycin and also in solid, crystalline, non-dissolved form. Crystalline natamycin is sparingly soluble in water and the finely divided solid natamycin crystals will be deposited on the surface together with the water and the dissolved natamycin. A preferred aqueous natamycin suspension for spraying purposes contains natamycin in a concentration of 250 to 7000 mg, more preferably 1000 to 4000 mg natamycin per litre of water.

A very small amount of natamycin is required to provide the desired protection against spoilage by moulds and yeasts. A deposited amount of from 1 to 10 µg natamycin per cm^2 of the surface of the baked product has been found to comprise an effective amount. It is of course possible to add more than the required amount of natamycin to the product. A higher amount than the above mentioned will certainly also be effective against mould and yeast growth and as natamycin has no bad taste, the product so protected would still be perfectly edible.

The natamycin should be sprayed homogeneously on all outer surfaces of the baked product so as to protect the product all over. Natamycin has a very low tendency for migration in the product and will not spread far from the point of deposition. In order to provide a homogeneous deposition the spraying equipment should be carefully selected. The natamycin based spray suspension is preferably delivered by a spinning disc, pneumatically operated spray gun or any other suitable spraying system that is capable of delivering a small but consistent and accurate spray volume over a

given surface area. The volume of the water based natamycin suspension sprayed onto the product should preferably be kept to the minimum level that will allow an even surface coverage. The natamycin deposited on the surface of the bakery product should, however, be effective in keeping the fine bakery product mould free even when stored for 2 weeks or more.

After spraying, the baked product is packaged into a protective envelope, which is preferably made of a transparent material such as a plastic film or box to allow the presumptive buyer to view the product and be tempted by it. The films are generally of a moisture proof material to prevent the moist baked product from drying and loosing its softness during the several weeks of storing

The following examples illustrate the invention.

Reference Example Natamycin in mooncakes

Mooncakes are traditional bakery products that are baked and eaten in large numbers once a year in China to celebrate a mid-Autumn festival. The cakes consist of a thin outer layer of pastry covering a variety of paste type fillings that are moulded into intricate shapes prior to baking. The outside of the cake is covered with an egg glaze and part cooked at 200°C –210°C for 15 minutes before a second coating of egg glaze is applied ready for the final bake of 10 minutes at the same temperature. Large-scale production, sale and storage of mooncakes begin in the period leading up to the festival and mould problems can occur on the surface of these products prior to consumption.

Two trial production runs of mooncakes containing lotus bean paste were made to test the efficacy of direct natamycin addition prior to baking for preserving these bakery products. The cakes were packed into individual clear plastic bags with no other preservative.

For the first production a range of four increasing natamycin levels (20, 25, 30 and 35 ppm) were mixed into the raw pastry dough for four small separate batches prior to glazing and baking. For the second production, nothing was added to the dough but an increasing range of the same four 20-35 ppm natamycin levels were added to the egg glaze that was applied to the cake surface after the first bake but before the second and final baking stage. Control cakes with no natamycin addition were also prepared.

Representative samples from each batch were assayed for residual natamycin in 10 g samples of the surface pastry layer. Good levels of residual natamycin activity were detected in the surface pastry of all samples from both production runs but despite this the growth of spoilage moulds still appeared on the surface of all of the natamycin treated cakes within 20 –24 days of manufacture.

Example 1 Natamycin on the surface of fine bakery

Muffins are flour-based non yeast-leavened fine bakery goods prone to surface spoilage due to growth of moulds and yeasts. Their water activity a_w is typically about 0.85.

Muffins were prepared according to a standard recipe with no added preservative in the dough. Shortly after baking, individual muffins were sprayed whilst still warm with a choice of four different spray treatments:

1 – water only (control).

2 – water containing 8% added salt.

3 – water containing 4g per litre of Natamax™ lactose (containing 50% natamycin) obtainable from Danisco A/S.

4 – water containing 8g per litre of Natamax™ lactose.

Spraying was done using a pneumatic hand-held spray gun with integral reservoir. The reservoir was regularly shaken during spraying to ensure that the small, undissolved crystals of natamycin stayed in suspension. Each muffin was sprayed evenly over all surfaces with a minimum volume of finely adjusted spray.

After cooling, the sprayed muffins were packed into heat sealed clear polythene bags with 8 to 10 muffins per treatment per bag. Initial samples were selected and tested for water activity, pH and surface natamycin concentration. Unopened bags of muffins were put for shelf life evaluation at 25°C and examined daily for signs of surface mould or yeast growth.

The surface natamycin determination was performed as follows: The surface area of the muffins was calculated to be 150 cm^2. Individual muffins were shaved and the total surface material added to 100 ml high purity methanol and shaken for 1 hour. 50 ml of high purity water was added and the solution then filtered through a 0.2 μm membrane filter. The natamycin content was determined by HPLC analysis for the whole surface and then divided by 150 to give a result in μg of natamycin per cm^2 of muffin surface.

Results from analysis of the initial samples are shown in Table 1. As expected, the highest level of residual natamycin was detected on the muffins sprayed with the highest concentration of Natamax™ suspension (treatment 4).

Table 1: Initial analysis results

Treatment	Residual natamycin μg/cm^2	Water activity (Aw)	pH
1) Water only	< 1	0.839	9.39
2) 8% salt	< 1	0.854	9.45
3) 4 g/l Natamax™	2.7	0.864	9.18
4) 8 g/l Natamax™	4.5	0.851	9.25

Results from the incubation study of the packs of muffins at 25°C are given in table 2. Packs of control muffins sprayed with water only or with the 8% salt in water were regarded as spoiled after only 5 and 11 days respectively due to the growth of clearly visible surface mould growth. In contrast

to this, all of the packs of muffins sprayed with the two treatment levels of Natamax™ suspension were found to be completely free of any surface mould growth for a total incubation period of 70 days, after which time the incubation was stopped.

Table 2: Results after incubation at 25°C

Treatment	Number of days in incubation at 25°C	Spoilage due to mould growth
1) Water only	5 days	All spoilt
2) 8% salt	11 days	All spoilt
3) 4 g/l Natamax™	70 days	None spoilt
4) 8 g/l Natamax™	70 days	None spoilt

At the end of the 70 day incubation period, triplicate samples of the unspoiled muffins from Natamax™ spray treatments 3 and 4 were assayed for surface natamycin levels, water activity (Aw) and pH. The results from these final analyses are given in Table 3. Residual natamycin was still detectable at similar levels to those of the initial sample for the higher concentration spray treatment 4 and at reduced levels for the lower concentration spray treatment 3.

Table 3: Final analysis results

Treatment	Residual natamycin $\mu g/cm^2$	Aw (muffin outside)	pH (muffin outside)	Aw (muffin inside)	pH (muffin inside)
3) 4 g/l Natamax™	0.7	0.85	6.97	0.85	8.15
	0.7	NT	NT	NT	NT
	0.7	NT	NT	NT	NT
4) 8 g/l Natamax™	4.2	0.83	6.21	0.84	7.91
	3.6	NT	NT	NT	NT
	4.8	NT	NT	NT	NT

NT = Not tested

The above results clearly demonstrate the excellent preservative efficacy of natamycin, when sprayed onto the surface of this relatively high moisture flour based baked product, in preventing or delaying spoilage due to surface growth of yeast and moulds

Example 2 Natamycin on the surface of fine bakery

Muffins were prepared according to the same standard recipe as in Example 1. Individual muffins were subjected to one of three treatments with a minimum of 50 muffins per treatment.

1 – No spray (control)

2 – Water only spray (control)

3 – Water containing 4.2 g per litre of Natamax™ SF (sugar free containing 87% natamycin) obtainable from Danisco A/S.

The muffins were sprayed shortly after baking whilst still warm. Spraying was done with a pneumatic hand-held spray gun with integral reservoir. The reservoir was regularly shaken during spraying to ensure that the small, undissolved crystals of natamycin stayed in suspension. The approximate minimum volume of spray required to evenly cover the whole surface of a muffin was measured and a concentration of 4.2 g per litre of Natamax™ SF calculated to target a spray delivery of 5 μg of natamycin per cm^2 of muffin in treatment 3. Each muffin was sprayed evenly over all surfaces.

After cooling, the sprayed muffins were packed into individual, heat sealed, clear plastic bags. One sample was selected from each of the two control treatments and five samples from the Natamax™ SF spray treatment and tested for water activity, pH, yeast and mould count at 25°C and residual surface natamycin levels by HPLC analysis. Forty unopened muffins per treatment were put for shelf life evaluation at 25°C and examined daily for signs of surface mould or yeast growth.

Results from analysis of the initial samples are shown in Table 4. The residual natamycin levels detected on the muffins sprayed with the Natamax™ SF suspension were found to be close to the target level of 5 μg per cm^2.

Table 4: Initial analysis results

Treatment	Residual natamycin μg/cm^2	Water activity (muffin outside)	Water activity (muffin inside)	Mould at 25°C/g	pH
1) No spray (control)	< 0.2	0.855	0.867	< 50	8.55
2) Water only (control)	< 0.2	0.863	0.877	< 50	8.65
3) 4.2 g/l Natamax™ SF	5.1	0.863	0.879	< 50	8.27
	4.3	NT	NT	< 50	NT
	3.4	NT	NT	< 50	NT
	3.8	NT	NT	< 50	NT
	5.8	NT	NT	< 50	NT

NT = Not Tested

Results from the incubation study of the 40 muffins per treatment are given in Table 5. The first non-spray control sample (treatment 1) developed surface mould growth after only 7 days giving a mould free shelf life of only 6 days. The first water sprayed control sample (treatment 2) developed surface mould growth after only 10 days. In contrast to this, all of the 40 muffins sprayed with

Natamax™ SF suspension remained completely free of any surface mould growth for a total incubation period of 68 days, after which time the incubation was stopped. Thus, in this trial experiment, a natamycin surface spray treatment of approximately 5 μg/cm^2 increased the mould-free shelf life of muffins at 25 °C from 6 days to at least 68 days.

Table 5: Results after incubation at 25°C

Days incubated at 25°C	Number showing mould growth / Number under incubation		
	Treatment 1 No spray control	Treatment 2 Water only control	Treatment 3 4.2 g/l Natamax™ SF
6	0/40	0/40	0/40
7	1/40	0/40	0/40
9	2/40	0/40	0/40
10	3/40	1/40	0/40
14	5/40	1/40	0/40
17	6/40	2/40	0/40
23	6/40	3/40	0/40
24	7/40	4/40	0/40
63	8/40	4/40	0/40
68	8/40	4/40	0/40

The above examples 1 and 2 clearly demonstrate the preservative efficacy of natamycin when sprayed on the outer surface of fine bakery products having a water activity above 0.8, which are susceptible to surface spoilage by moulds and yeasts during storage. Based on the description and examples a person skilled in the art is able to apply the invention to a wide variety of fine bakery goods.